SCHOOL OF
CIVIL ENGINEERING

INDIANA
DEPARTMENT OF HIGHWAYS

JOINT HIGHWAY RESEARCH PROJECT

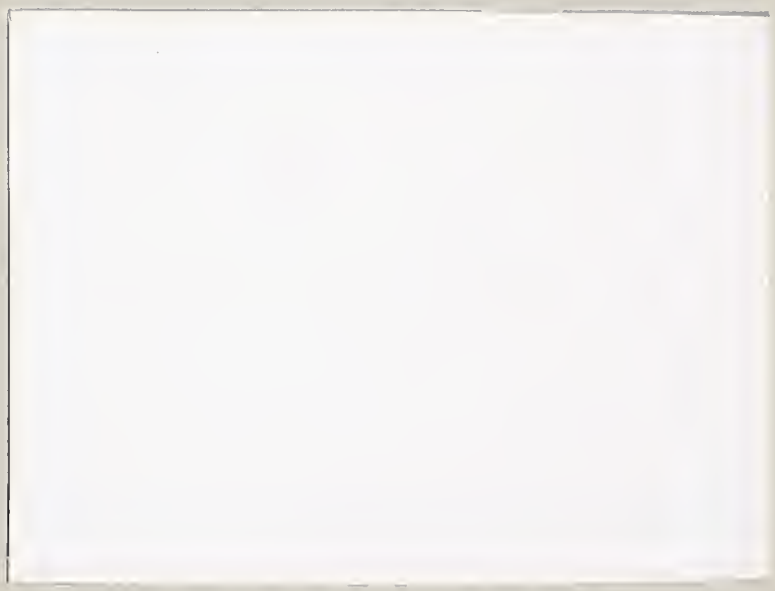
FHWA/IN/JHRP-82/22

TECHNIQUES TO INCREASE SURVIVAL OF NEW HIGHWAY PLANTINGS (ROOT REGENERATION PORTION)

FINAL REPORT

B. C. Moser
R. J. Kelly

PURDUE UNIVERSITY



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Final Report

TECHNIQUES TO INCREASE SURVIVAL OF NEW HIGHWAY PLANTINGS (ROOT REGENERATION PORTION)

To: Robert L. Eskew, Chairman
Joint Highway Research Project
Advisory Board

From: Harold L. Michael, Director
Joint Highway Research Project

December, 1982
Revised: March, 1984 and
June, 1985

Project: C-36-48H

File: 9-5-8

The attached final report on the root regeneration portion of the HPR Part II study "Techniques to Increase Survival of New Highway Plantings" is submitted as the final product of this three-part project. Messrs. B. C. Moser and R. J. Kelly have authored this report. All work on this portion as well as on other portions of this study was conducted by personnel of the Horticulture Department, School of Agriculture.

The major conclusions are applicable to those species of trees that are difficult to establish on the highway roadside. The use of auxin preplant treatments to hasten the regeneration of a new root system of trees should be considered for coarse rooted bare root material that is known to be difficult to transplant.

The Final Report is presented to the IDOH and FHWA as completion of all requirements of this Study.

Harold L. Michael
Director

HLM/tb

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FINAL REPORT

TECHNIQUES TO INCREASE SURVIVAL OF NEW HIGHWAY PLANTINGS (ROOT REGENERATION PORTION)

by

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Joint Highway Research Project

File 9-5-8

Project C-36-48H

Prepared as Part of an Investigation

Conducted by

Joint Highway Research Project
Engineering Experiment Station
Purdue University

in cooperation with the

Indiana State Highway Commission

and the

U.S. Department of Transportation
Federal Highway Administration

The contents of this report reflect the views of the authors who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the Federal Highway Administration. The report does not constitute a standard, specification, or regulation.

Purdue University
West Lafayette, Indiana
December 1982
Revised March 1984
& June 1985

TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
FHWA/IN/JHRP-82/22		

4. Title and Subtitle	5. Report Date
TECHNIQUES TO INCREASE SURVIVAL OF NEW HIGHWAY PLANTINGS (ROOT REGENERATION PORTION)	December 1982 Revised: March 84 & June 85
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
B. C. Moser and R. J. Kelly	JHRP-82-22

9. Performing Organization Name and Address	10. Work Unit No.
Joint Highway Research Project Civil Engineering Building Purdue University West Lafayette, Indiana 47907	11. Contract or Grant No. HPR-1(21), Part II
12. Sponsoring Agency Name and Address Indiana Department of Highways State Office Building 100 North Senate Avenue Indianapolis, Indiana 46204	13. Type of Report and Period Covered Final Report, Root Regeneration Portion
	14. Sponsoring Agency Code CA 384

15. Supplementary Notes
Conducted in cooperation with U. S. Department of Transportation, Federal Highway Administration as a portion of HPR Part II Study titled, "Techniques to Increase Survival of New Highway Plantings.

16. Abstract

Root regeneration in several species of plants was enhanced by a five minute preplant root soak in from 1,000 to 3,000 mg/liter aqueous solutions of indolebutyric acid (IBA). Positive responses were confined to coarse rooted difficult-to-transplant species such as tulip tree, red oak, black walnut, white oak, black tupelo and redbud. Fibrous rooted species such as silver maple and sugar maple did not respond to the auxin treatment and two species of pine were adversely affected. Root soaks were the most effective technique utilized to apply auxin.

Tulip tree (Liriodendron tulipifera L) was selected as a model system for indepth studies on root regeneration under controlled conditions as well as field sites. Root regeneration was greater in the spring than in the fall but was not effected by photoperiod. There was a strong interaction between root regeneration and subsequent shoot growth. Plant survival following transplanting in the field was enhanced in IBA treated plants compared to untreated plants.

Root regeneration and subsequent shoot growth was influenced by soil temperature with little growth occuring below 10°C. IBA accelerated root regeneration only when soil temperature was above 21°C.

Carbohydrate reserves (primarily starch) in the taproot were depleted as new roots formed. This depletion was accelerated by IBA treatment and reached critically low levels at the highest concentrations of IBA used.

17. Key Words	18. Distribution Statement
Tree planting, tulip tree, root regeneration, IBA, auxin	No restrictions. This document is available to the public through the National Technical Information Service, Springfield, VA 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	52	

Form DOT F 1700.7 (8-69)

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HIGHLIGHT SUMMARY

Several species of plants responded positively to IBA (indolebutyric acid) treatment by different techniques of application. The most effective form of treatment was a five minute root soak in from 1000-3000 mg/liter IBA. Greater root number and root elongation were observed in one year old seedlings of tulip tree, red oak, white oak, black walnut, black tupelo and redbud following IBA treatment. Positive response was confined to coarse rooted difficult to transplant species. Fibrous rooted species such as silver maple and sugar maple did not respond to the auxin treatment and the roots of two species of pine were adversely affected. Root soaks in aqueous solutions of IBA required less manipulation of plants and seemed most efficient relative to ease of treatment when compared to other techniques of application.

Root regeneration of tulip tree (Liriodendron tulipifera L.) seedlings was greater when transplanted in the spring than in the fall. Indolebutyric acid (IBA) applied to the roots increased root regeneration with the greatest response at 1000 mg/liter in the spring and 3000 mg/liter in the fall. Root regeneration and shoot growth increased as chilling hours increased from 0 to 1680 hours; 1008 hours of storage at $2^{o}C$ was sufficient to fulfill the chilling requirement. Photoperiod did not directly influence root regeneration in either the spring or the fall. There was an interaction of root regeneration and shoot growth of shoot pruned plants with season, IBA treatment, and level of pruning. Decreasing the light intensity of full sunlight reduced root regeneration and shoot growth. Applying IBA improved root initiation over untreated plants at all light intensities, but subsequent root growth decreased with decreased light. Survival percentage and vegetative growth of field planted tulip trees was greater when plants were pretreated with IBA root soaks.

Root regeneration and shoot growth of tulip tree increased as the temperature of the growing environment increased from 10° to 21°C. IBA applied at the rate of 1000 mg/liter to the roots increased root regeneration and shoot growth at temperatures of 15.5° and 21°. When air temperature was constant 21° and soil temperature was varied from 10° to 21°, root regeneration and shoot growth of untreated plants increased as soil temperature increased. IBA significantly increased root regeneration only after soil temperature was raised to 21°. This suggests that cold soils may inhibit root regeneration of bare root trees on highway sites and treatment with IBA will not overcome the effects of low soil temperatures.

Endogenous levels of starch and sucrose measured in the taproot of dormant tulip tree decreased during the course of root initiation and elongation. Applications of IBA to the taproot stimulated root regeneration and resulted in larger decreases in starch and sucrose levels in the taproot. Excessive depletion of reserve carbohydrates in roots can be triggered by high levels of IBA. Levels of starch, sucrose, and reducing sugars in the stem tissue declined during root regeneration, but there was no significant difference between IBA treatments. These results indicate that root regeneration in dormant tulip tree is dependent on the carbohydrates contained primarily in the taproot with starch the major source. Trees that have depleted carbohydrate supplies due to accelerated root regeneration from high levels of IBA treatment may be sensitive to stress conditions often found at highway sites. Because of this, IBA should be used with caution.

INTRODUCTION

The loss of newly transplanted woody plants is a major problem in landscape and highway sites. During transplanting a substantial portion of the rapidly elongating tissue of the root system is lost or removed. Under these conditions a plant may be severely stressed since nutrient and water uptake is limited. The ability for a plant to initiate adventitious roots following transplanting is essential for its establishment and growth.

Root initiation and growth of woody plants is greatly influenced by species, environment, and cultural practices. Investigations have shown that plants exhibit species and varietal differences with respect to root development when grown under the same enviromental conditions. Auxin, applied to the roots of several species, will enhance root regeneration and growth. However, the response of a plant to auxin treatment appears to be dependent on other factors governing their growth.

The research presented in this report centers around a series of preliminary experiments carried out with several species of plants leading to more detailed experiments using the tulip tree as a model system. Each section of the report has its own introduction section compatible with the material being covered.

The overall objectives of the research presented in this report were:

1) to determine the effectiveness of various root growth stimulating chemicals on regrowth of roots from transplanted trees and shrubs,

2) to investigate alternative techniques for applying root growth stimulating chemicals to trees and shrubs,

3) to determine the impact of increased root regeneration on survival and subsequent growth of transplanted trees and shrubs, and

4) to determine what place, if any, treatments that stimulate root regeneration have on highway plantings.

PRELIMINARY EXPERIMENTS

Introduction

The successful establishment of trees and shrubs in a landscape setting has been a serious problem in all landscape sites, including those along highways. When woody plants are dug and transplanted they undergo severe stress since much of the root system is lost and water uptake by remaining roots is limited. The capacity to resume root growth rapidly is often the critical requirement to survival of transplanted trees and shrubs (1). Thus it is the first month or two after transplanting that determines the potential success of a highway planting.

Work in the past has been done on fertilizers, mulches, etc. but no attempt has been made to take advantage of chemicals which are known to enhance root growth and their potential application to plant material used in highway plantings. These chemicals are in a class called "Auxins" and are commonly used to stimulate root initiation during cutting propagation (2).

Several researchers have demonstrated that these chemicals will also enhance root regeneration and growth of transplanted maple (3), oak (4), and pecan (5). Hence it is clear that "auxins" do have the capacity to stimulate root growth and may well be part of the answer to getting plants off to a quicker start in highway plantings. Recent work on oaks (6) has shown that "auxins" can effectively be used to increase transplant survival and the practice is being recommended to nurserymen at this time.

The research was undertaken on this project to provide a basis for use of "auxin" treatments on plants in highway plantings. This may well aid in more rapid establishment and cut down on losses of trees and shrubs installed along highways in Indiana and other states.

Objectives include the following:

1. Investigate the feasibility of using synthetic auxins other than IBA to stimulate root regeneration.
2. Determine the feasibility of enhancing root regeneration in several difficult-to-transplant species.
3. Examine alternate methods of applying auxins to roots.

Methods and Results

Relative Effectiveness of Several Auxin-like Chemicals

Roots of year old transplants of black walnut, redbud, and white oak were soaked for five minutes in several different concentrations of IBA, NAA, 2,4-D, 2,4,5-T, 2,4,5-TP, and CPA. Treated plants were potted and held for six weeks after which new root growth was measured. Of the chemicals studied, only IBA (Indolebutyric acid-potassium salt) and NAA (Napthalene acetic acid-potassium salt) were effective. Typical results are presented in Table 0-1.

IBA root soaks resulted in approximately four times as many new roots with no detrimental effect on root length. NAA was the next most active chemical in terms of new root numbers but it also stunted root growth. All other growth regulators gave minimal stimulation in terms of root numbers and stunted subsequent root growth. Similar results were achieved with white oak and redbud.

These results coupled with our previous work with scarlet oak suggest that IBA is the only suitable auxin for use in stimulating root regeneration.

IBA Enhanced Root Regeneration in Several Species

Roots of one year old seedlings of several species of difficult-to-transplant as well as easy to transplant trees were soaked in various concentrations of IBA for five minutes. Plants were potted and held for six weeks in the greenhouse. Effects on root regeneration are illustrated in Table 0-2.

Table 0-1. Effects of various auxin-like growth regulators on root regeneration and growth of year old black walnut seedlings.

Treatment	Optimum concentration (ppm)	Number of new roots	Average new root length (cm)
Control	-	3.0	6.0
IBA	1000	11.4	6.1
NAA	1000	7.7	4.4
2,4-D	100	4.0	3.4
2,4,5-T	100	4.6	3.8
2,4,5-TP	10	2.4	4.4
CPA	1000	5.2	3.7

Table 0-2. Effect of IBA on root regeneration of selected plant species*.

Plant	IBA Effect		
	Positive effect	Negative effect	No effect
Tulip Tree	X		
Silver Maple			X
Sugar Maple			X
Black Walnut	X		
Black Tupelo	X		
White Oak	X		
Red Oak	X		
White Pine		X	
Austrian Pine		X	
Flowering Quince			X
Redbud	X		

*In all cases where positive effects were seen, optimum concentrations of IBA root soaks were at 1,000 and 3,000 mg/l.

Results on fibrous rooted tree species such as maple were inconclusive as no effect could be observed following treatment. Positive effects of greater root regeneration occurred only on those species with coarse root systems such as tulip tree, black walnut, black tupelo, white oak, red oak, and redbud. The most promising responses occurred with tulip tree, and it was chosen as the species to investigate in further detail. As the Indiana State tree, it would be meaningful if techniques could be worked out for greater ease of establishment. For unknown reasons, the use of IBA and similar chemicals on two pine species not only failed to produce more roots but actually contributed to significantly higher mortality rates. After two experiments confirming this, it was decided to discontinue further research on the evergreens. Since experiments with balled and burlapped plants were planned with pines, they were also deleted from the study.

Alternate Methods of Applying Auxins to Roots

Several methods of applying auxins to roots were investigated. They include the following:

1. Root dip or soak in auxin solution.
2. Auxin impregnated toothpicks inserted crossways into the root.
3. A gel dip as a carrier for auxin.
4. Auxin impregnated string tied around roots.

Results of various treatments are presented in Table 0-3.

Several methods of applying the chemical were as effective as root soaks. However, root soaks were the easiest to accomplish as plants could be treated in groups. Other treatments had to be done on a single plant basis and required significant labor. Because of this the root soak method was chosen for all further treatments in our studies.

Table 0-3. Effects of various methods of applying IBA for enhanced root regeneration of year old white oak seedlings*.

Treatment	Number of new roots
Control	2.3
Root soak - 3000 ppm	6.4
Toothpick - 1000 ppm	6.0
Gel dip - 3000 ppm	9.5
String - 10000 ppm	7.6

*Selected data from best treatments and auxin concentrations - similar results with redbud and tulip tree.

Conclusions

Preliminary experiments determined the following:

1. IBA was the most effective auxin studied. All other auxins had adverse effects on root elongation.
2. Only difficult to transplant coarse rooted trees responded favorably to auxin treatment. Of these, tulip tree was the most responsive.
3. Pine trees were adversely effected by auxin treatment to the roots.
4. The root soak method was the treatment of choice when both labor required and response obtained were considered.

The above conclusions indicate that auxin pretreatment at the nursery can be utilized to enhance root regeneration on difficult to transplant species. The tulip tree, (Liriodendron tulipifera L.), is a good model system to use in further detailed studies of root regeneration.

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ROOT REGENERATION OF *Liriodendron tulipifera* L. IN RESPONSE TO AUXIN, STEM PRUNING AND ENVIRONMENTAL CONDITIONS

Abstract

Root regeneration of tulip tree (*Liriodendron tulipifera* L.) seedlings was greater when transplanted in the spring than in the fall. Indolebutyric acid (IBA) applied to the roots increased root regeneration with the greatest response at 1000 mg/liter in the spring and 3000 mg/liter in the fall. Root regeneration and shoot growth increased as chilling hours increased from 0 to 1680 hours; 1008 hours of storage at 2°C was sufficient to fulfill the chilling requirement. Photoperiod did not directly influence root regeneration in either the spring or the fall. There was an interaction of root regeneration and shoot growth of shoot pruned plants with season, IBA treatment, and level of pruning. Decreasing the light intensity of full sunlight reduced root regeneration and shoot growth. Applying IBA improved root initiation over untreated plants at all light intensities, but subsequent root growth decreased with decreased light.

Introduction

Most of the absorbing root surface of bare-root seedlings is either removed in the digging process or desiccates during handling and winter storage. The ability of bare-root seedlings to initiate new roots quickly after transplanting is essential for establishment and growth. Successful establishment requires that adventitious roots be initiated on older roots which have undergone secondary differentiation.

Root regeneration potential varies with species, the physiological and developmental stage of the plant, and the environment during root formation (15). Several species have a limited capacity to regenerate roots in the

dormant season when grown under favorable conditions (5,12). In these plants root regeneration is related to active shoot growth. If shoots are dormant, root regeneration will be limited unless the chilling requirements are met (3,4,20,21). Many woody plants exhibit a seasonal periodicity in root regeneration with its potential being maximum when the chilling requirements have been fulfilled and declining through early summer as shoot growth increases (5,11,18).

Auxin applied exogenously to the roots of woody plants stimulates root initiation (16) and enhances establishment. Root regeneration was promoted by the application of IBA in transplanted pear (13), oak (12), pecan (16), and various evergreens (5).

Tulip tree transplants poorly in the fall and only spring transplanting is recommended (1). The root system, as with other species of the Magnoliaceae, consists of a fleshy taproot which is coarsely branched and, thus, requires adventitious root development after transplanting. Little information is available on the root regeneration and early growth of transplanted tulip tree. The objectives of this study were to determine effects of 1) season, 2) shoot dormancy, 3) auxin application, 4) photoperiod control, 5) shoot pruning, 6) disbudding and 7) shading on the root regeneration of tulip tree.

Materials and Methods

Greenhouse Studies

Plant material. One-year-old seedlings of tulip tree were obtained from the Jasper-Pulaski State Nursery, Medaryville, Indiana. Seedlings, about 0.75 cm stem diameter, were selected for uniformity; shoots were pruned to 15 cm and roots were pruned to 10 cm with all secondary roots removed unless otherwise noted.

Seedlings were transplanted into a pasteurized growing medium in 1.9 liter waxed cardboard cartons and grown in the greenhouse at 25° (day)/15°C (night) temperatures. Expect as noted, plants were grown under long days consisting of natural daylengths and a 4 hr light break from incandescent lamps (3.5 $\mu E\ m^{-2}\ sec^{-1}$ measured at pot height), from 9:00 PM to 1:00 AM. The growing medium consisted of 1 soil, 2 sphagnum peat moss, and 2 perlite (by volume) with a pH of 6.2. The growing mixture was amended with 0.9 (kg/m^3) superphosphate, 0.6 KNO_3, 0.6 $MgSO_4$, 5.3 agricultural limestone, and 0.075 trace elements. Additional fertilization consisted of 200 mg/liter N and K (pH 6.8) applied at each watering. Plants were harvested 8, 10, and 12 weeks after planting (November 17, 1979) in the fall, except where noted. Seedlings grown in the spring were harvested 6, 8, and 12 weeks after planting (March 23, 1980).

Auxin. Roots were soaked for 5 min in aqueous solutions of the potassium salt of IBA (ICN Nutritional Biochemicals, Cleveland, Ohio) at 0, 300, 1000, 3000, or 10,000 mg/liter. All IBA concentrations were tested in spring and fall.

Chilling. Seedlings obtained in the fall were stored at 2°C and removed after 0, 336, 672, 1008, 1344, 1680 hr. Shoots and roots were pruned to a uniform size, transplanted, and grown in the greenhouse. Shoot and root growth of each group was measured after 10 weeks.

Photoperiod. Plants were subjected to photoperiods of 8-, 12-, and 18-hr. Photoperiod was extended with incandescent lighting of 3.5 $\mu E\ m^{-2} sec^{-1}$ at the end of an 8-hr day. The 8-hr day length was maintained by placing black cloth over the plants at 4 PM and removing it at 8 AM. Auxin was applied to the roots at the rate of 0 or 1000 mg/liter IBA.

Shoot pruning. Seedlings selected for a uniform taproot and a shoot length of 60 ± 5 cms were shoot pruned to lengths of 2.5, 15, 30, and 60 cm. The roots were pruned to a 10 cm taproot and treated with either 0 or 1000 mg/liter IBA.

Disbudding. Seedlings were root and shoot pruned as described and divided into 2 groups. One group had all the visible buds removed at the time of pruning, and any buds which began to elongate during the experiment were removed. The second group consisted of non-disbudded plants. The roots were treated with either 0 or 1000 mg/liter IBA.

Shading. Plants were pruned as described and grown under 0, 20, 47 or 73% shade in the spring. Reduced light intensities were provided by structures enclosed with polypropylene shading material. Roots were treated with either 0 or 1000 mg/liter IBA.

Field Studies

Seedlings were shoot and root pruned and treated with auxin as described above and planted in the field in the fall (November 19, 1979) and spring (May 8, 1980). Fall transplants were harvested May 10, 1980, while spring transplants were harvested 6, 8, and 12 weeks after planting.

Three-year-old trees (6.3 cm caliper) were obtained from Hobb's Nurseries, Indianapolis, Indiana. Shoots were pruned to reduce the total shoot area by 1/3, roots were pruned to 20 cm from the main taproot. Trees were treated with 0, 1000, and 3000 mg/liter IBA, planted May 10, 1980, and harvested one year later.

All field plots were irrigated as needed to maintain adequate soil moisture. Though this procedure would not normally be done on the roadside, the effect of water stress needed to be eliminated from the study.

Experimental Design and Data Collection

Greenhouse studies were arranged in a split plot with treatment as the whole plot and time as the subplot. Field plots were arranged in a randomized complete block. The number and dry weight of new roots and shoots was determined. Callus present on the base of the tap root was used to indicate seedling viability when shoot and root growth were absent. Treatment differences were maximum at 12 weeks and only this data is presented. Data are averages of 9 replicates per treatment taken at specified harvest dates. The 3-year-old plants were rated visually during the growing season on a scale from 1 to 7 with 1 (death), 4 (moderate dieback), and 7 (actively growing).

Results

Greenhouse Studies

Season-auxin interaction. IBA applied to the roots of tulip tree seedlings in the fall promoted root regeneration at concentrations above 300 mg/liter (Figure I-1). The number of new roots was greatest at 10,000 mg/liter while the root dry weight peaked at 3000 mg/liter IBA. Although there were numerous roots at 10,000 mg/liter, they were less branched than those at 3000 mg/liter IBA.

Shoot dry weight of fall transplants was highest at 1000 mg/liter IBA. Although there were a few actively-growing shoots at 8 weeks, by 12 weeks there was an average of 1 active shoot per plant. The correlation between root dry weight and shoot dry weight was low ($r^2 = 0.44$).

Root regeneration was higher and the response to IBA was greater in the spring than in the fall. Root number was greatest at the highest IBA concentration, but the weight of new roots peaked at 1000 mg/liter IBA (Figure I-1). IBA appeared to be toxic at 10,000 mg/liter since there was a 33% mortality rate.

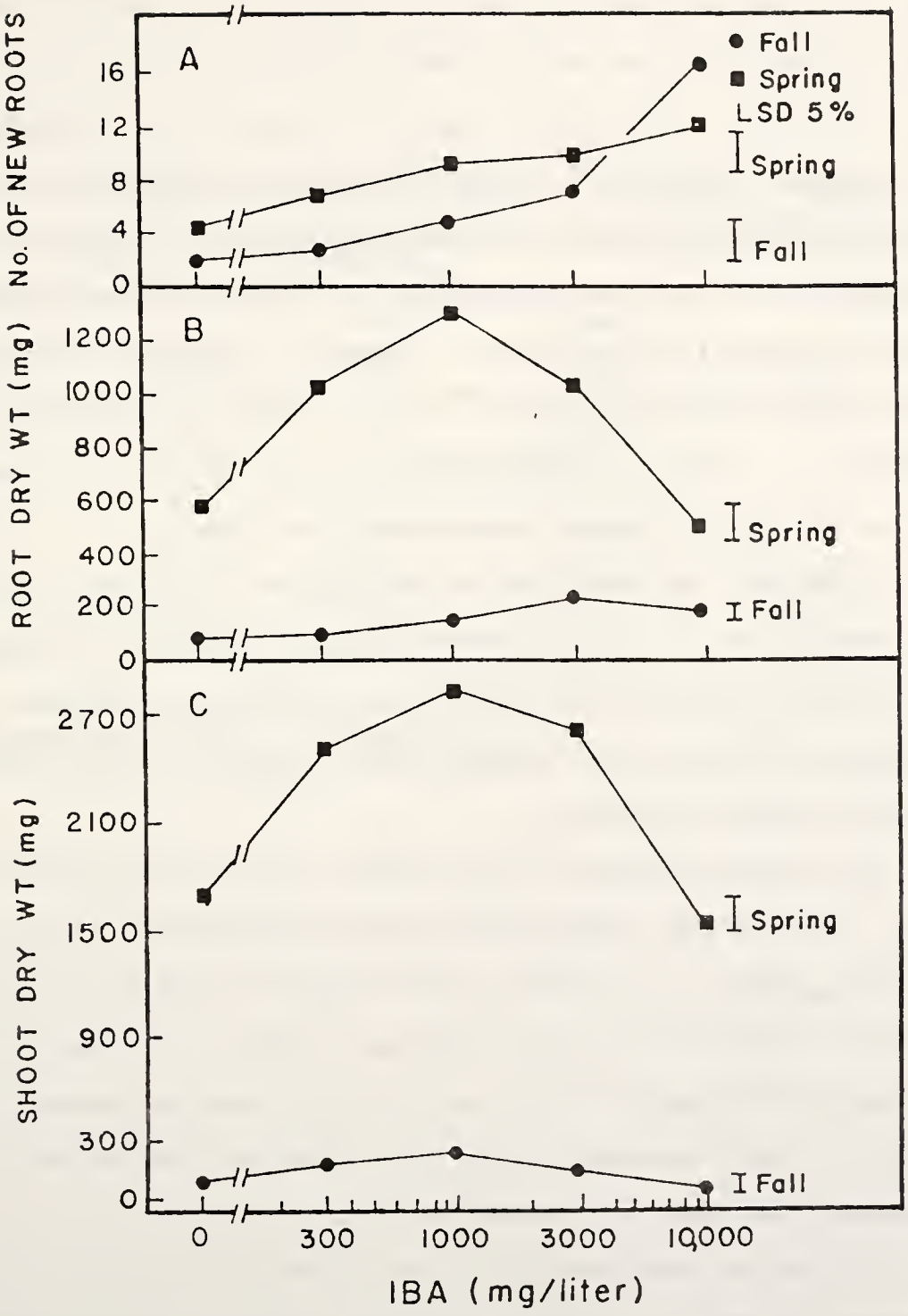

Figure I-1. The influence of IBA on the number of roots (A), root dry weight (B), and shoot dry weight (C) of tulip tree seedlings grown in the greenhouse in the fall and spring and harvested 12 weeks after transplanting.

Bud break in spring transplants was evident 2 weeks after planting, and most plants had initiated shoot growth after 6 weeks. Shoot dry weight was maximum at 1000 mg/liter IBA. Root dry weight correlated well with shoot dry weight ($r^2 = 0.79$). The rate of 1000 mg/liter IBA should not be exceeded when applying IBA to bare root tulip trees to be planted on the roadside.

Chilling. Shoot growth and root regeneration increased as chilling time surpassed 336 hr (Table I-1). Uniform bud break did not occur until plants had received 1008 hr of chilling suggesting that the chilling requirement for tulip tree is between 1008 and 1344 hr. Increased chilling time was highly correlated with shoot activity ($r^2 = 0.97$). Plants stored for 1008 hr broke bud within 2 weeks and elongated with shoot growth and root regeneration similar to non-stored plants transplanted in the spring.

Photoperiod. Daylength did not influence root regeneration of fall-grown seedlings (Table I-2). Auxin improved the root response at all daylengths, but the response between photoperiods was not significantly different. The 8-hr photoperiod inhibited shoot growth, while longer days did not promote shoot growth of spring transplants.

In the spring, daylength did not affect root regeneration with or without auxin. Shoot growth increased with increasing photoperiods of 12- to 18-hr.

Shoot pruning. In the fall, root and shoot dry weight was maximum when shoots were unpruned (60 cm) and auxin was applied to the roots (Figure I-2). As stem length decreased from 60 to 2.5 cm as a result of pruning, root and new shoot dry weight decreased. Plants not treated with IBA had the greatest root dry weight when shoots were pruned to 15 cm.

The effect of shoot pruning was most evident in the spring. Maximum root regeneration and shoot growth of untreated plants occurred when shoots were

Table I-1. The influence of chilling time on the number of roots, root dry weight and shoot dry weight of tulip tree seedlings measured on plants grown in the greenhouse and harvested 10 weeks after transplanting.

Chilling time (hr)	No of new roots	Root dry wt (mg)	Shoot dry wt (mg)	Budbreak (%)
0	.8 a[z]	24 a	29 a	11
336	.6 a	33 a	41 a	33
672	1.7 b	148 b	256 b	44
1008	1.2 b	181 bc	270 b	78
1344	2.4 c	195 c	515 c	100
1680	3.3 c	262 d	606 c	100

[z] Mean separation by LSD at the 5% level.

Table I-2. The influence of daylength and the interaction with IBA application on the number of roots, root dry weight and shoot dry weight of tulip tree seedlings. Measurements were taken on plants in the greenhouse and harvested at 12 weeks.

Treatment		No. roots		Root weight (mg)		Shoot weight (mg)	
		IBA concn		IBA concn		IBA concn	
Season	Daylength (hr)	0 mg/liter	1000 mg/liter	0 mg/liter	1000 mg/liter	0 mg/liter	1000 mg/liter
Fall	8	1.0 a[z]	4.2 b	26.6 a	68.6 b	0 a	0 a
	12	1.6 a	4.2 b	30.8 a	65.0 b	28.6 a	57.3 a
	18	1.2 a	5.0 b	21.4 a	74.0 b	7.7 a	96.0 b
Spring	8	2.2 c	6.2 d	169.8 c	439.5 d	749.4 c	972.0 c
	12	3.0 c	6.4 d	210.1 c	503.9 d	990.0 c	1552.2 d
	18	2.8 c	5.0 d	209.5 c	307.9 d	1448.8 d	1562.5 d

[z] Mean separation by LSD at the 5% level.

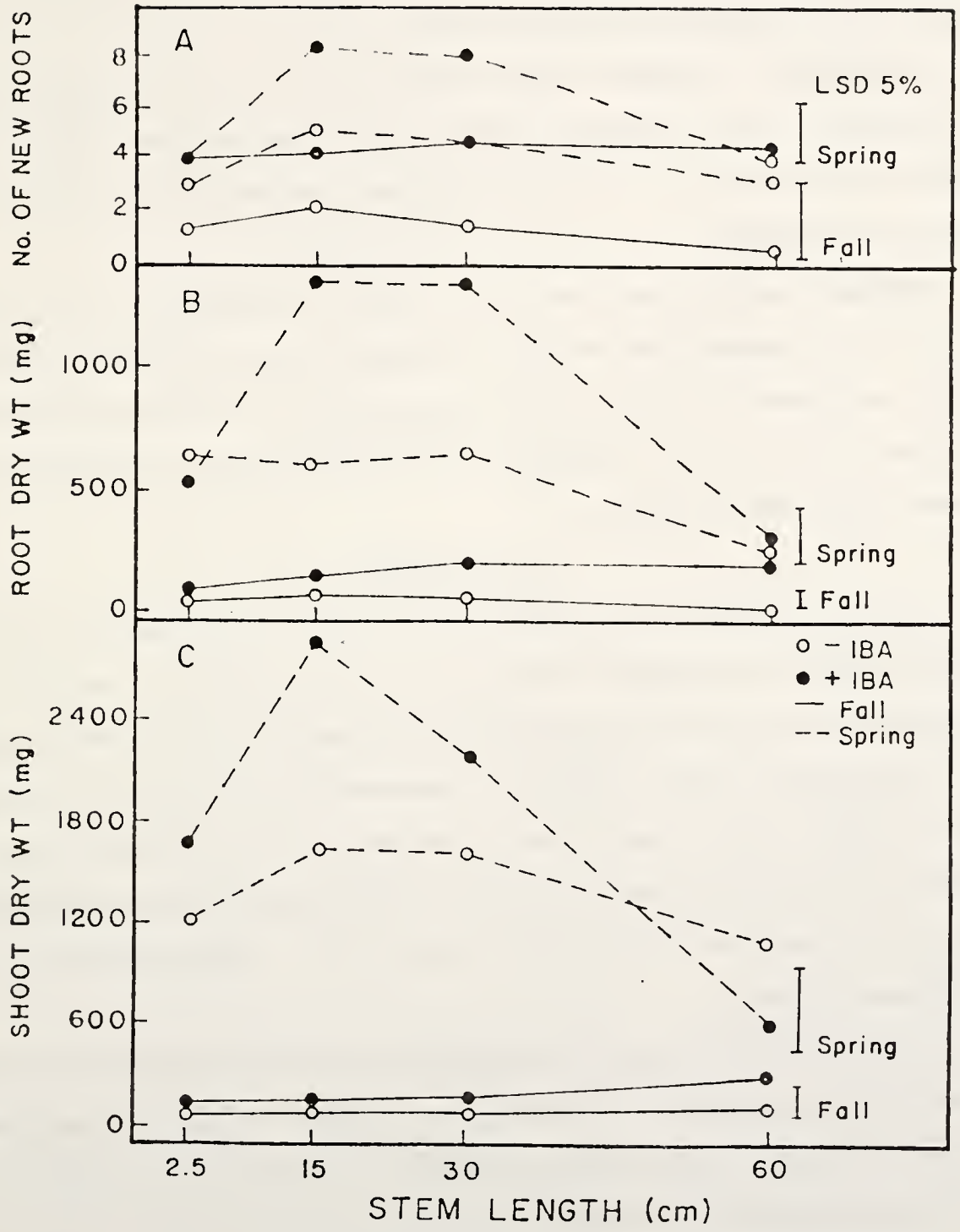

Figure I-2. The influence of shoot pruning and the interaction with IBA application on the number of new roots (A), root dry weight (B), and shoot dry weight (C) of seedlings grown in the greenhouse in the spring and the fall and harvested 12 weeks after transplanting.

pruned to 15 or 30 cm. The response to pruning in auxin treated plants was similar; although, means were higher than untreated plants with shoot growth greatest when plants were pruned to 15 cm.

Disbudding. Bud removal in the fall reduced the root dry weight but not the number of roots regenerated regardless of auxin treatment (Table I-3). Most seedlings died as a result of disbudding in the spring, and those that did survive grew poorly.

Shading. Light intensity had little effect on the number of roots regenerated, but the highest level of root regeneration and shoot growth resulted when plants were grown in full sun and declined to a low at 73% shade (Figure I-3). Applying IBA to the roots increased root and shoot growth over untreated plants except at the 73% shade level.

Field Studies

Auxin was ineffective in promoting root initiation and growth of field grown seedlings transplanted in the fall. All the plants were dead with no evidence of root regeneration when plants were harvested the following spring.

In the spring, auxin enhanced root regeneration as it had in the greenhouse studies (Figure I-4). The largest increases in root and shoot dry weight occurred with 1000 and 3000 mg/liter IBA. Plants treated with 10,000 mg/liter IBA often showed toxic effects with death of some seedlings (33%) as in greenhouse studies.

Trees treated with IBA had a greater rate of survival and improved root and shoot growth as compared to untreated plants (Table I-4). Root and shoot growth was greatest and the number of plants that survived was highest when trees were treated with 3000 mg/liter IBA.

Table I-3. The influence of bud removal and the interaction with IBA treatment on the number of roots, root dry weight and shoot dry weight of tulip tree seedlings grown in the greenhouse and harvested 12 weeks after transplanting.

Season	Pruning Treatment	No.of new roots		Root dry wt (mg)		Shoot dry wt (mg)	
		0 mg/liter	1000 mg/liter	0 mg/liter	1000 mg/liter	0 mg/liter	1000 mg/liter
Fall	Disbudded	1.3 a[z]	5.6 b	22.0 a	106.4 c	--	--
	Intact Shoot	2.0 a	6.5 b	64.0 b	223.8 d	56.2 a	225.5 b
Spring	Disbudded	1.0 k	1.7 k	3.1 k	4.9 k	--	--
	Intact Shoot	4.2 m	8.8 n	589.2 m	1015.9 n	1719.4 m	1657.7 n

[z] Mean separation by LSD at the 5% level.

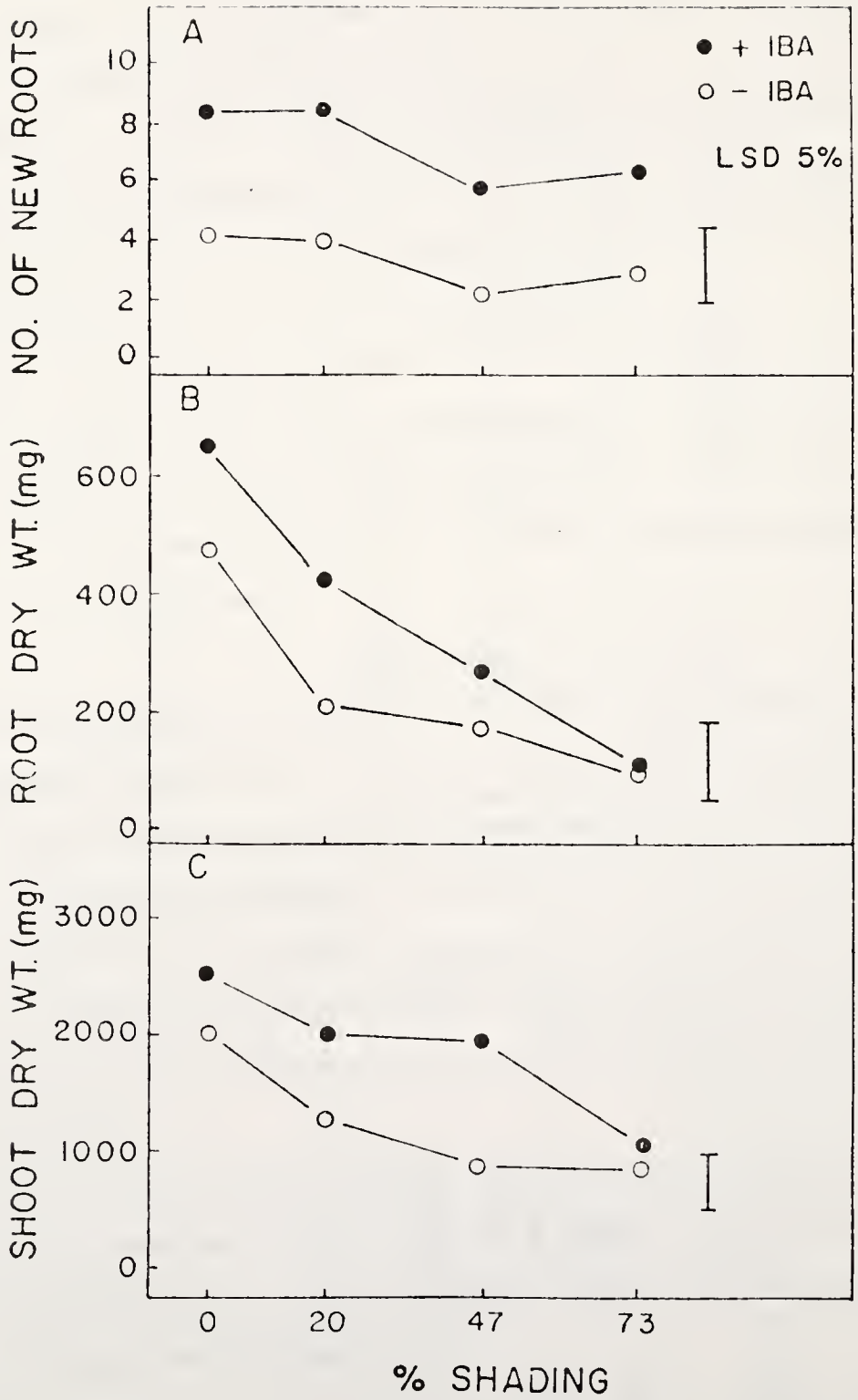

Figure 1-3. The influence of shading and the interaction with IBA application on the number of new roots (A), root dry weight (B), and shoot dry weight (C) of seedlings grown in the greenhouse in the spring 12 weeks after transplanting.

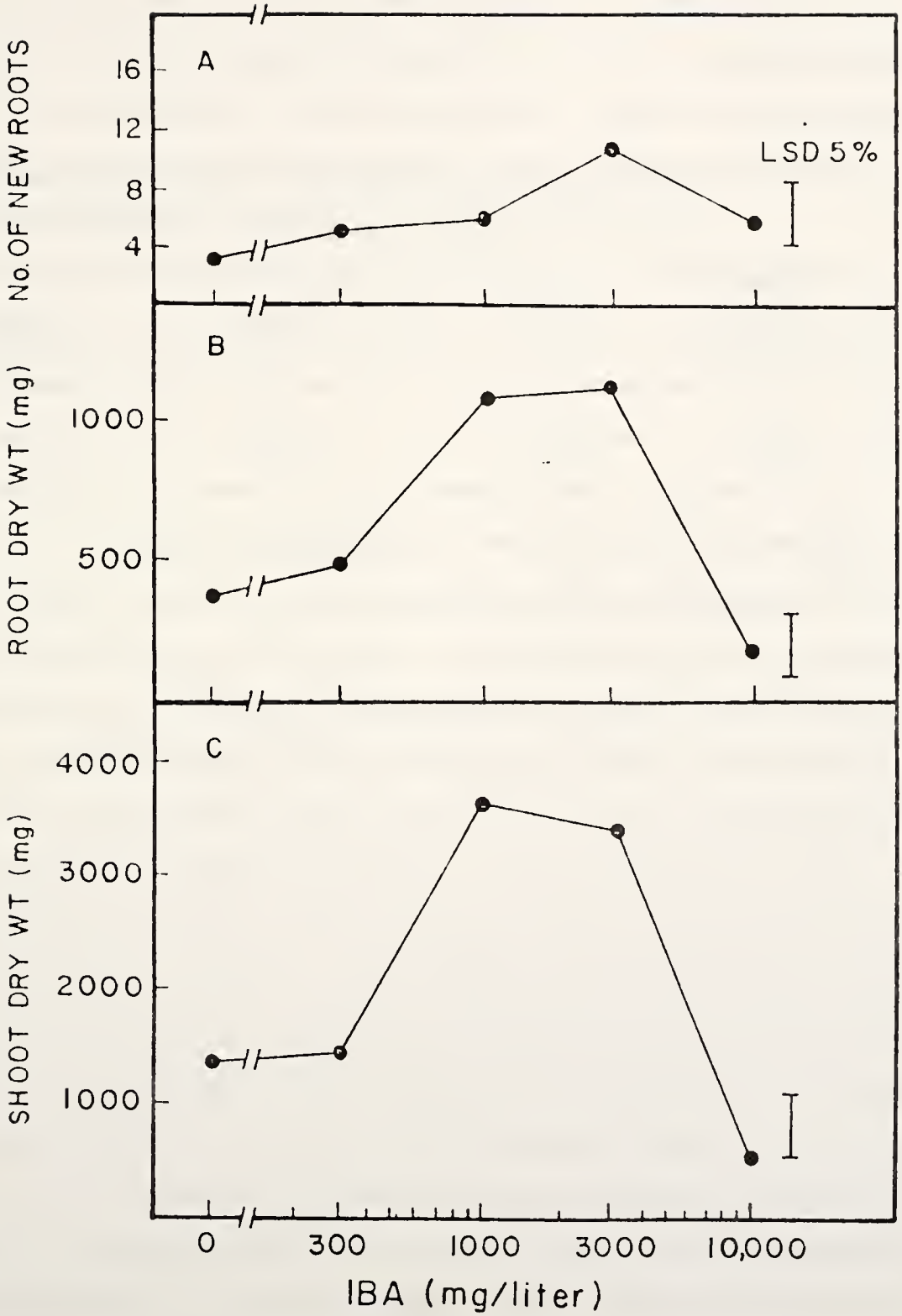

Figure I-4. The influence of IBA on the number of roots (A), root dry weight (B), and shoot growth of tuplip tree seedlings grown in the field and harvested 12 weeks after transplanting.

Table I-4. The influence of IBA application on the root regeneration and survival of 6.3 cm caliper plants outplanted in the spring and harvested one year later.

IBA concn (mg/liter)	Root dry wt (g)	Survival (%)	Visual rating[z]
0	0.6 a[y]	44	2.0
1000	7.6 ab	56	2.9
3000	31.4 c	100	5.1

[z] Based on scale of 1 (death) to 7 (actively growing)

[y] Mean separation of LSD, 5% level.

Discussion

Tulip tree exhibits a seasonal periodicity in root regeneration with increased activity in the spring as compared to fall. This seasonal difference appears to be due primarily to active shoot growth. When plants were grown in the spring, budbreak occurred rapidly with active shoots present well in advance of the emergence of new roots.

IBA enhanced root regeneration in the fall and the spring relative to control plants with the greatest increases occurring in the spring. The success of IBA in stimulating root regeneration is due primarily to an increase in the number of roots regenerated rather than earliness of root initiation. This is evident since roots in all treatments were initiated and well established by 8 weeks following treatment. The best overall root regeneration and shoot growth occurred by treating seedlings with 1000 mg/liter in the spring and 3000 mg/liter in the fall. The seasonal difference in optimum concentration may be related to the activity of vegetative buds and the influence shoot growth has on root initiation and growth. When plants were treated with 10,000 mg/liter, large numbers of roots were initiated, but they did not elongate or branch as compared to those treated with lower concentrations of IBA. This effect has been observed in cuttings treated with high concentrations of IBA (6). Trees treated with 3000 mg/liter IBA in the spring yielded the greatest increase in root growth and plant survival. The difference in the response to IBA of seedlings versus trees may be due in part to the woody character of the roots of the more mature plants.

Although it was possible to enhance root initiation in the fall by auxin treatment, the subsequent growth of these roots was limited by the lack of vigorous shoot growth. In the case where no shoot activity occurred, the new

roots which were initiated develop from the mobilization of reserves within the plant. In contrast, active shoot growth occurred prior to root regeneration in the spring which would contribute photosynthate for developing roots. At times, plants would contain a short actively growing shoot without the initiation of roots at 6 weeks which is additional evidence for shoot growth preceeding root regeneration. However, if a seedling had not regenerated roots by 12 weeks it usually did not survive. Thus, shoot growth appears essential for optimum root regeneration, and the critical period for establishment appears to be between 6 and 12 weeks following transplanting under favorable growing conditions.

This seasonal periodicity of rooting has been seen in several other species of woody plants (11,12). The root regeneration and bud break of woody plants are often related and are influenced by the hours of chilling required to fulfill the chilling requirements (3,4,20,21). Pretreatment with IBA has been shown to improve root regeneration of woody plants (13,16), including tulip tree (7). However, IBA could not alter the seasonal periodicity in root regeneration (3).

Root regeneration and shoot growth increased with increasing chilling time. A cold treatment of between 1008 to 1344 hours appears sufficient to allow uniform root and shoot growth under forcing conditions. Because of this chilling requirement, root regeneration of plants transplanted outdoors does not appear likely until after the plants have received sufficient cold, and environmental conditions permit growth.

While short days inhibited shoot growth, dormant plants did not initiate significant shoot growth under longer photoperiods until the chilling requirement had been fulfilled. In the spring 12- or 18-hr photoperiods

promoted shoot growth with the 18-hr photoperiod promoting the greatest shoot length (21.6 cm, 18-hr vs. 9.6 cm, 12-hr). However, photoperiod did not appear to effect root regeneration directly. The onset of dormancy in tulip tree has been shown to be influenced by short photoperiod with long days promoting active growth which could be maintained for at least 18 months when plants were grown under favorable temperatures (19). In this study, photoperiod could not substitute for the cold requirement in promoting shoot and root growth.

Stem pruning is a common practice used to reduce the shoot length of seedlings in an effort to compensate for the loss of roots. It is generally thought that pruning will help maintain a balanced shoot/root ratio (20), stimulate lateral root formation and/or aid in efficient handling and transplanting. In this study, all plants were pruned to a single 10 cm tap root to place transplants under severe stress in regard to the necessity for root regeneration. Mild shoot pruning in the spring enhanced root regeneration of untreated plants but had little effect on seedlings grown in the fall. Reducing the shoot length to 30 or 15 cm in the spring improved root regeneration and shoot growth.

In the fall, plants treated with auxin did not benefit from pruning and, in fact, developed the largest root system when the shoot was unpruned. This effect of pruning may be due to the need for reserve materials contained in the shoot for root development. In the spring, significant growth increases were achieved by moderate shoot pruning with or without IBA application. In either case, auxin treatment will improve the root regeneration and shoot growth as compared to untreated plants. These results agree with those of Sterling and Lane (17) who found that maintaining a balanced shoot/root ratio improved the performance of tulip tree seedlings transplanted in the spring and grown in the field.

The presence of buds is necessary for optimum new root development in the fall and spring. IBA could promote root regeneration when applied to disbudded plants in the fall. However, plants do not achieve levels that occur on seedlings with an intact shoot. Shoot activity is essential for root regeneration and plant survival in the spring. The poor performance of disbudded plants with or without applied auxin supports the relationship between bud activity and root regeneration.

Increased shading reduced root regeneration and shoot growth. Auxin improved root and shoot growth; however, the amount of growth declined with increased shading. As the light intensity was decreased shoots became increasingly elongated or stretched, as is typical for plants grown at low light levels. Reducing light intensities for stock has been shown to influence rooting of cuttings taken from these plants (2,9,14). The physiological response to shading appears related to production and storage of photosynthates and/or the levels of endogenous hormones contained in these plants (14). In our study, root initiation was not affected by light intensity, but root and shoot growth were reduced by shading. The manner in which shading influences photosynthate production and/or hormone levels during root regeneration needs investigation.

Understanding the seasonal periodicity of root regeneration and the influence of shoot dormancy on root regeneration will be useful in adapting methods to enhance the survival and growth of transplanted tulip trees. It should be noted that the plants used in this study were selected from a seedling population which resulted in variability of response regardless of treatment. This variability in growth of tulip tree has been noted by Kellison (8) and may be useful in selecting plants based on rates of root regeneration.

Fall transplanting does not appear to be feasible for tulip tree in northern latitudes. The need for chilling and the onset of winter resulting in lower soil temperatures most likely prevent root regeneration prior to spring. The response of tulip tree to auxin application in the fall in regions where temperatures are moderate and dormancy is not as well defined should be investigated. In the spring, moderate pruning and a localized pretreatment with auxin will improve root regeneration and establishment of both bare-root seedlings and trees and will result in a plant with a branched and compact root system which increases the root surface area.

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THE INFLUENCE OF TEMPERATURE AND AUXIN ON ROOT REGENERATION OF SEEDLINGS OF *Liriodendron tulipifera* L.

Abstract

Root regeneration and shoot growth of tulip tree (*Liriodendron tulipifera* L.) increased as the temperature of the growing environment increased from 10° to 21°C. Indolebutyric acid (IBA) applied at the rate of 1000 mg/liter to the roots increased root regeneration and shoot growth at temperatures of 15.5° and 21°. When air temperature was constant 21° and soil temperature was varied from 10° to 21°, root regeneration and shoot growth of untreated plants increased as soil temperature increased. IBA significantly increased root regeneration only after soil temperature was raised to 21°. This suggests that plantings on the roadside be made only after soil temperatures have warmed in the spring.

Introduction

Early establishment of woody plants after transplanting is critical for their survival. Most of the absorbing roots of bare-root transplants are lost during the digging process or desiccate during handling and winter storage. For successful establishment of bare-root transplants roots must be initiated on tissues which have undergone secondary differentiation.

Tulip tree is difficult to transplant, and only spring transplanting is recommended (2). The root system, as with other species of the Magnoliaceae, consists of a coarsely branched, fleshy taproot and, thus, requires adventitious root development after transplanting. Root regeneration in tulip tree is seasonally periodic with greater rooting in spring than in fall. IBA applied to the roots enhanced root regeneration but did not change the seasonal periodicity (5). Optimum soil temperatures for root initiation and growth have

been reported for various woody species (6), but, little information is available on root initiation at low temperature characteristic of the dormant season when woody plants are transplanted. In this study, we investigated the effects of auxin, and soil and air temperature on the root regeneration and early growth of tulip tree.

Materials and Methods

Plant material. One-year-old bare-root seedlings of tulip tree were obtained in the fall, from the Jasper-Pulaski State Nursery, Medaryville, Indiana. Plants were held at 2°C until the start of the experiments on March 8, 1980 and March 15, 1981. Seedlings, about 0.75 cm stem diameter, were selected for uniformity, shoots were pruned to 15 cm, and roots were pruned to 10 cm with all secondary roots removed.

Varied air and soil temperature. The study was conducted utilizing 3 reach-in growth chambers (Sherer-Gillett, Marshall, Michigan, model 512-37) equipped with 82% input wattage of 135 W Cool White fluorescent lamps of very high output (VHO) and 18% from 60 W frosted incandescent lamps. The irradiation at pot height was 500 $\mu E\ m^{-2} sec^{-1}$ measured with a Lambda LI-185 Quantum, Radiometer (Lambda Instrument Co., Lincoln, Nebraska). The light period was 16-hr and dark period 8-hr, with an abrupt change. Plants were grown at air and soil temperatures of either 10°, 15.5° or 21°C.

The roots of seedlings were soaked for 5 min in aqueous solutions of IBA (ICN Nutritional Biochemicals, Cleveland, Ohio) at 0 or 1000 mg per liter. Seedlings were transplanted into a pasteurized growing medium in 1.9 liter waxed cardboard cartons. The growing medium was of 1 soil: 2 sphagnum peat moss: 2 perlite (by volume) with a pH of 6.2. The potting mixture was amended with 0.9 (kg/m^3) superphosphate, 0.6 KNO_3, 0.6 $MgSO_4$, 5.3 agricultural

limestone, and 0.075 trace elements. Additional fertilizer was applied at each watering at the rate of 200 mg/liter N and K (pH 6.8).

Varied soil temperature. The study was conducted in a growth chamber under day length and irradiation conditions as described above. Plants were subjected to a constant air temperature of 21°C and soil temperatures of 10°, 15.5°, or 21°. The soil temperatures of 10° and 15.5° were achieved by growing plants in a 30 cm high insulated wooden chamber 57 x 87.5 cm. Copper tubing was placed in the soil between the rows of plants at 10 and 20 cm below the soil surface. The tubing carried coolant circulated from a refrigerated bath (Forma Scientific, Marietta, Ohio, model 2067) which reduced soil temperatures to the desired levels. The 21° soil temperature, the same as ambient air temperature, was obtained by growing plants in a similar root box without temperature modifying materials. The soil temperatures were monitored at 3, 10, and 20 cm using a multipoint recorder (Honeywell Corp., Fort Washington, Pennsylvania, model 112). The temperature remained relatively constant throughout the box except at the top 3 cm which increased about 2° due to the influence of air temperature. The soil mixture, plant maintenance, and auxin treatment were the same as for experiment 1.

Data collection and experimental design. The number and dry weight of new roots, and dry weight of new shoot tissue produced after transplanting were determined. Callus present on the base of the taproot was used to indicate seedling viability when shoot and root growth was absent. Plants were harvested at 6, 8, and 12 weeks after transplanting in experiment 1 and after 6 and 12 weeks in experiment 2. Treatment differences were maximum at 12 weeks and only this data is presented. The experimental design was a split plot with temperature as the whole plot and treatment as the subplot. Data are averages of 9 replicates per treatment.

Results

Varied air and soil temperature. Increasing the air and soil temperature from 10° to 21°C increased root regeneration and shoot growth for both IBA treated and untreated plants (Fig. II-1). For the untreated group, root initiation was low at 10° with only 2 plants initiating roots after 12 weeks. About half of the seedlings initiated roots at 15.5° and most plants initiated roots at 21°. The dry weight of roots increased with temperature, and the greatest amount of root growth occurred at 21°.

IBA increased the number of roots over the untreated plants by 2.0 roots (12.7 mg dry weight) at 10°C; 3.5 roots (229.3 mg dry wt) at 15.5° and 5.8 roots (882.5 mg dry wt) at 21°. Regardless of treatment, root initiation and growth was low at 10°; however, applying IBA to the roots increased the number of plants regenerating roots from 2 to 6 at 10°. The number of plants regenerating roots at 15.5° and 21° was similar regardless of treatment; however, the means for root number and dry weight were higher when treated with IBA.

Generally, plants initiated shoot growth by 6 weeks, and this growth increased with time. Shoot dry weight increased as temperatures increased from 10°C to 21°. When plants were grown at 10°, shoots consisted of a few small leaves with low dry weight.

At 10°C shoot growth for IBA-treated plants was similar to untreated plants. Increasing the temperature to 15.5° or 21° and treating with IBA increased shoot dry weight over untreated plants. Shoot growth was maximum for plants treated with IBA and grown at 21° for all harvest dates tested.

Varied soil temperature. When air temperature was held constant and the soil temperature reduced, soil temperature affected root and shoot growth.

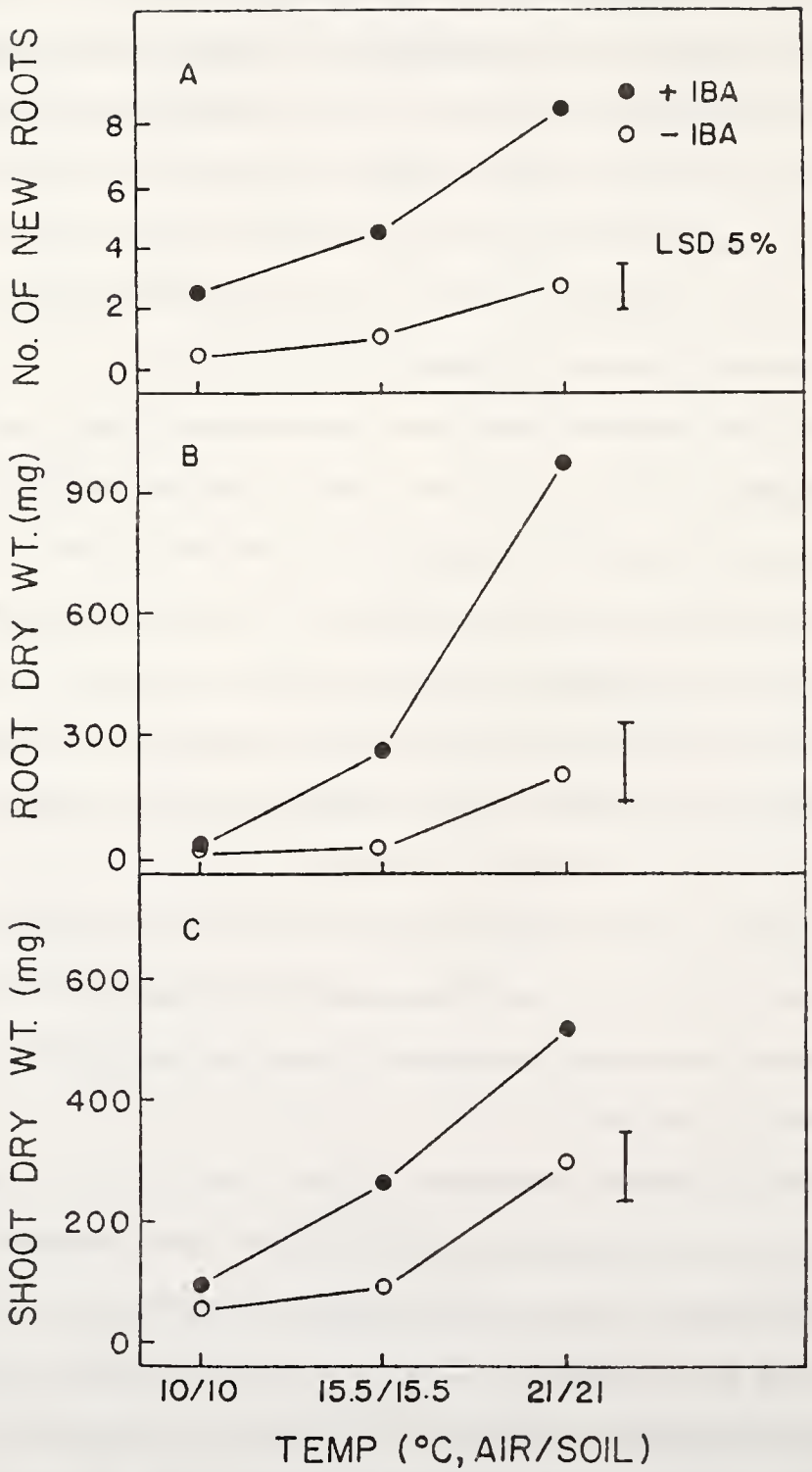

Figure II-1. The influence of constant air and soil-temperatures (10°, 15.5°, and 21°C) and IBA (0 and 1000 mg/liter) on the number of new roots (A), root dry weight (B), and shoot dry weight (C) of tulip tree seedlings 12 weeks after transplanting.

Increasing the soil temperature from 10°C to 21° increased root initiation, and root and shoot growth (Fig. II-2). Applying IBA to the roots did not improve root initiation at 10° as compared to untreated plants. The number of roots increased by 1.1 (98.5 mg dry wt.) at 15.5° and 3.1 (196.4 mg dry wt) at 21° over the untreated plants. Most seedlings had initiated shoot growth by 6 weeks, in many cases without the initiation of new roots. Shoot growth was similar for treated and untreated plants at 10° or 15.5°, but IBA significantly increased growth at 21°.

Discussion

Root regeneration and shoot growth of tulip tree increased as the temperature of the growing environment increased. Shoot growth was initiated soon after transplanting at all 3 temperatures, but the rate of growth and amount of shoot tissue was influenced by temperature. IBA increased the rate of root regeneration and shoot growth as temperature increased to 21°C with maximum growth at 21°.

Soil temperatures had significant effects on root growth when air temperature was held constant. These effects were more pronounced when plants were treated with IBA. Cold soil not only reduced root regeneration but reduced shoot growth even when air temperatures were high.

Various studies with several species indicate that rate of root and shoot growth is related to temperature of the growing environment (1,3,4,9,10). Maximum root regeneration of tulip tree resulted when plants were grown at 21°C which is within the range (20°-30°) observed for Pinus radiata (8).

Although high air temperatures occur in the early spring, soil temperatures remain low in the field. In West Lafayette, Indiana, the average soil temperature does not reach 21°C at a depth of 10.2 to 20.3 cm until after the first of

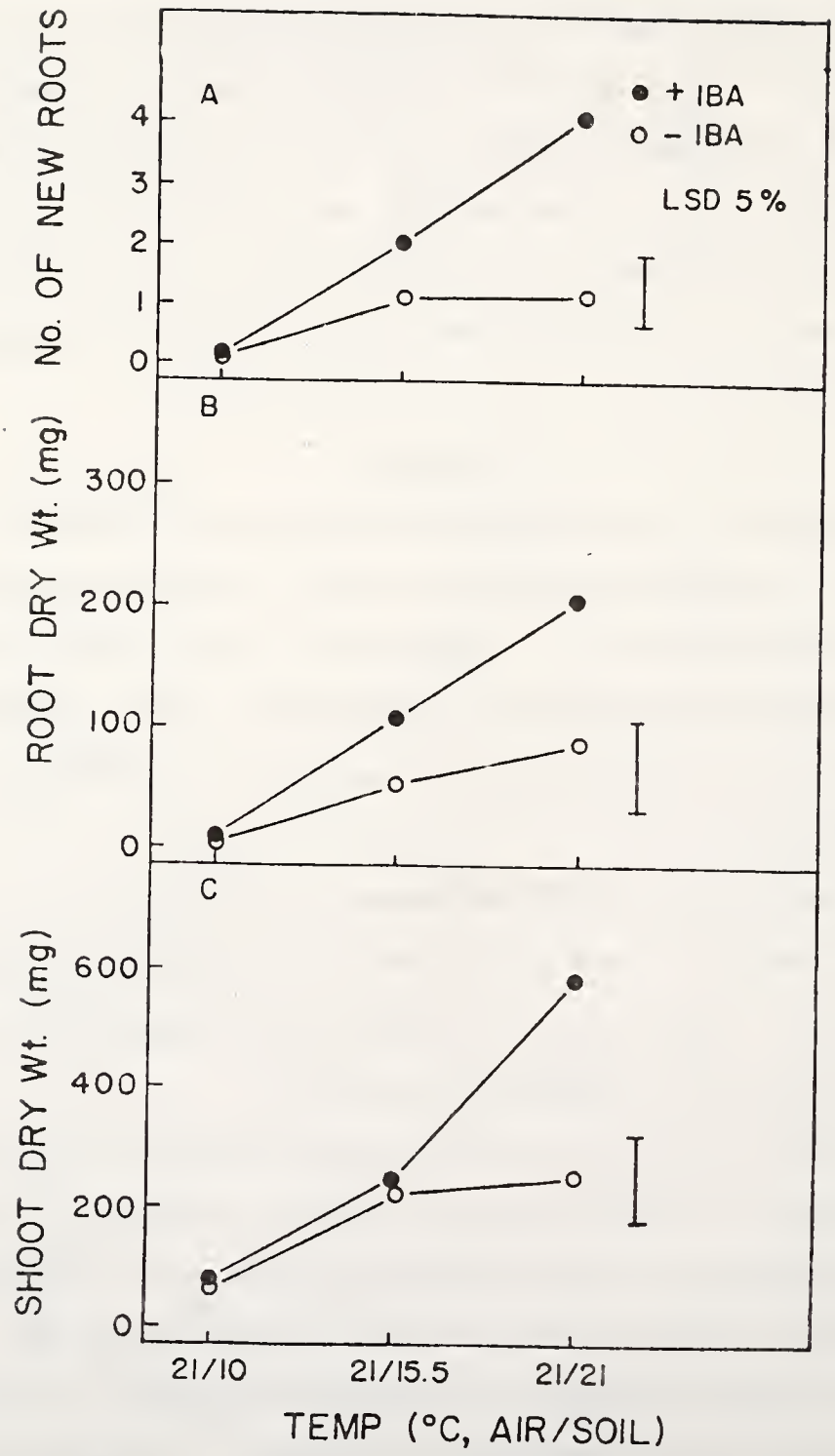

Figure II-2. The influence of constant air temperature (21°C), soil temperatures of 10°, 15.5°, and 21° and IBA (0 and 1000 mg/liter) on the number of new roots (A), root dry weight (B), and shoot dry weight (C) of tulip tree seedlings 12 weeks after transplanting.

June (7). This suggests that cool soil temperature may be an important factor limiting root regeneration and establishment of tulip tree transplanted in the early spring. IBA applied to the roots could compensate for lower temperatures by improving root regeneration and allowing more rapid development to enhance plant establishment.

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INFLUENCE OF IBA ON CARBOHYDRATE UTILIZATION DURING THE PROCESS OF ROOT REGENERATION IN DORMANT *Liriodendron tulipifera* L.

Abstract

Endogenous levels of starch and sucrose measured in the taproot of dormant tulip tree (*Liriodendron tulipifera* L.) decreased during the course of root initiation and elongation. Applications of indolebutyric acid (IBA) to the tap root stimulated root regeneration and resulted in larger decreases in starch and sucrose levels in the taproot. Reducing sugars tended to be higher in the taproot of those plants exhibiting increased rooting. Levels of starch, sucrose, and reducing sugars in the stem tissue declined during root regeneration, but there was no significant difference between IBA treatments. These results indicate that root regeneration in dormant tulip tree is dependent on the carbohydrates contained in the taproot with starch the major source. Only trees that have been stored properly should be used in highway plantings.

Introduction

In a previous study (5) we reported the seasonal periodicity of rooting and the influence of various environmental conditions on the root regeneration of tulip tree. Dormant seedlings initiated roots without shoot growth when grown in the greenhouse under short days (8-hr) and IBA applied to the roots enhanced root regeneration. The new roots initiated on dormant plants must develop from the mobilization of reserves. Carbohydrates, the major form of stored energy within the plant, are the chief constituents of cell walls, serve as the starting point for fat and protein synthesis, and are oxidized during respiration. Thus, the utilization of these components is directly related to root regeneration. The objective of this study was to examine the relationship between root regeneration and carbohydrates within the dormant seedlings.

Materials and Methods

Plant material. One-year-old seedlings of tulip tree were obtained from the Jasper-Pulaski State Nursery, Medaryville, Indiana. Plants were held at 2°C prior to the beginning of the study on November 29, 1981. Seedlings, about 0.75 cm stem diameter, were selected for uniformity. The shoots were pruned to 15 cm, and roots were pruned to 10 cm with all secondary roots removed. The roots were soaked for 5 min in aqueous solutions of the potassium salt of IBA (ICN Nutritional Biochemicals, Cleveland, Ohio) at 0, 1000, or 10,000 mg/liter.

Seedlings were transplanted into 1.9 liter waxed cardboard cartons, and grown in a greenhouse with thermostats set at 25° (day)/15°C (night). Plants were subjected to an 8-hr daylength achieved by placing black cloth over them from 4 pm to 8 am. The growing medium consisted of 1 soil, 2 sphagnum peat moss, and 2 perlite (by volume) with a pH of 6.2. The growing mixture was amended with 0.9 (kg/m^3) superphosphate, 0.6 KNO_3, 0.6 $MgSO_4$, 5.3 agricultural limestone, and 0.075 trace elements. Additional fertilization consisted of 200 mg/liter N and K (pH 6.8) applied at each watering.

Carbohydrate determinations. Tissue was sectioned (at 2°C), frozen in liquid N_2, lyophilized, and stored at -20°C until analyzed for carbohydrate content.

Lyophilized tissue was weighed, then ground to a powder using a Wig-l-bug (Crescent Dental Mfg. Co., Lyons, Illinois, model LP-60). Samples of 100 mg roots and 400 mg shoots were extracted in 80% ethanol at 70°C for 30 min and centrifuged at 12,062 x g for 10 min to remove insoluble materials. The pellet was re-extracted and centrifuged 2 additional times and retained for starch analysis.

The ethanol fractions were combined and brought to volume. Subsamples of this fraction were dried via vacuum evaporation and taken up in 50 mM sodium

acetate buffer (pH 4.7). Total sugars were determined by treating a fraction of this solution with 20 units of invertase (Sigma Chemical Co., St. Louis, Missouri) in acetate buffer for 45 min at 37°C; control samples were similarly treated with acetate buffer without invertase. The reducing sugar content was determined by the method outlined by Cherry (5). Sucrose content was calculated from the difference between invertase-treated and control samples.

The pellet was washed twice with 20% methanol and centrifuged between washes at 3000 x g for 20 min to remove soluble materials. Following the wash, samples were treated with 0.1% pronase (Calbiochem-Behring Corp., La Jolla, CA) in 50 mM Tris buffer with 10 mM $CaCl_2$ and trace HgI_2 (pH 8) for 12 hr at 37°C and then centrifuged at 3000 x g and washed twice with 20% methanol as described.

Starch was isolated by extracting the pronase-treated pellet with dimethyl sulfoxide (DMSO) for 12 hr, and centrifuged between extractions at 3000 x g for 20 min. DMSO extracts were combined, filtered through Whatman No. 52 paper under vacuum and brought to volume. The DMSO-dispersed starch was precipitated in 95% ethanol (1/3 by volume), centrifuged for 20 min at 3000 x g and dissolved in 0.1 N NaOH. Aliquots of this solution were tested for soluble sugars utilizing the anthrone reagent as outlined by Withan, Blaydes, and Devlin (10).

Data collection and experimental design. Seedlings were removed from the containers, and the number and dry weight (DW) of roots determined. Carbohydrate analysis was performed on 3 sections (proximal, medial, and distal) of the taproot, the stem, and newly initiated roots.

The experimental design was a split plot with treatment as the whole plot and time as the subplot. There were 9 plants per treatment, harvested at 0.2, 4, 6, and 8 weeks after transplanting. Three samples per treatment that best

represented the mean were selected for analysis. Root regeneration was monitored weekly for the first 4 weeks until root initiation was apparent. The data was analyzed by linear regression, with each curve representing the best fit by r^2 and F values.

Results

Root regeneration was promoted by IBA treatment with the greatest response at 10,000 mg/liter (Figure III-1) confirming data previously reported (6). Root initiation was apparent at 4 weeks with the majority of the new roots initiated on the distal portion of the root (Figure III-2).

The taproot contained about 22% starch per 100 mg DW which was 72% of total carbohydrates measured in the root at the beginning of the experiment. Starch levels declined with time and IBA treatment, but there was no significant difference between root sections. For the untreated plants, starch levels declined 19% (17.71 mg/100 mg DW) by 4 weeks and did not change significantly for the duration of the experiment. IBA applied at the rate of 1000 mg/liter resulted in a similar decline in starch compared to untreated plants up to 4 weeks, but declined an additional 19% to 12.26 mg/100 mg DW by 8 weeks. The greatest decline in starch resulted when seedlings were treated with 10,000 mg /liter IBA. By 4 weeks starch content for this treatment declined 44% to 12.59 mg/100 DW and declined an additional 26% to 6.53 mg/100 mg DW by 8 weeks.

Sucrose accounted for about 6.5% of the total dry weight of the taproot which was about 22% of the total carbohydrate measured. The level of sucrose declined with time and increased IBA treatment, and there was no significant difference between root sections. At week 0 the taproot contained about 6.52 mg/100 mg DW, and for untreated plants declined 51% to 3.21 mg/100 mg DW by 8 weeks. Seedlings treated with 1000 mg/liter IBA resulted in a more rapid rate of decline in sucrose levels as compared to untreated plants. Sucrose content

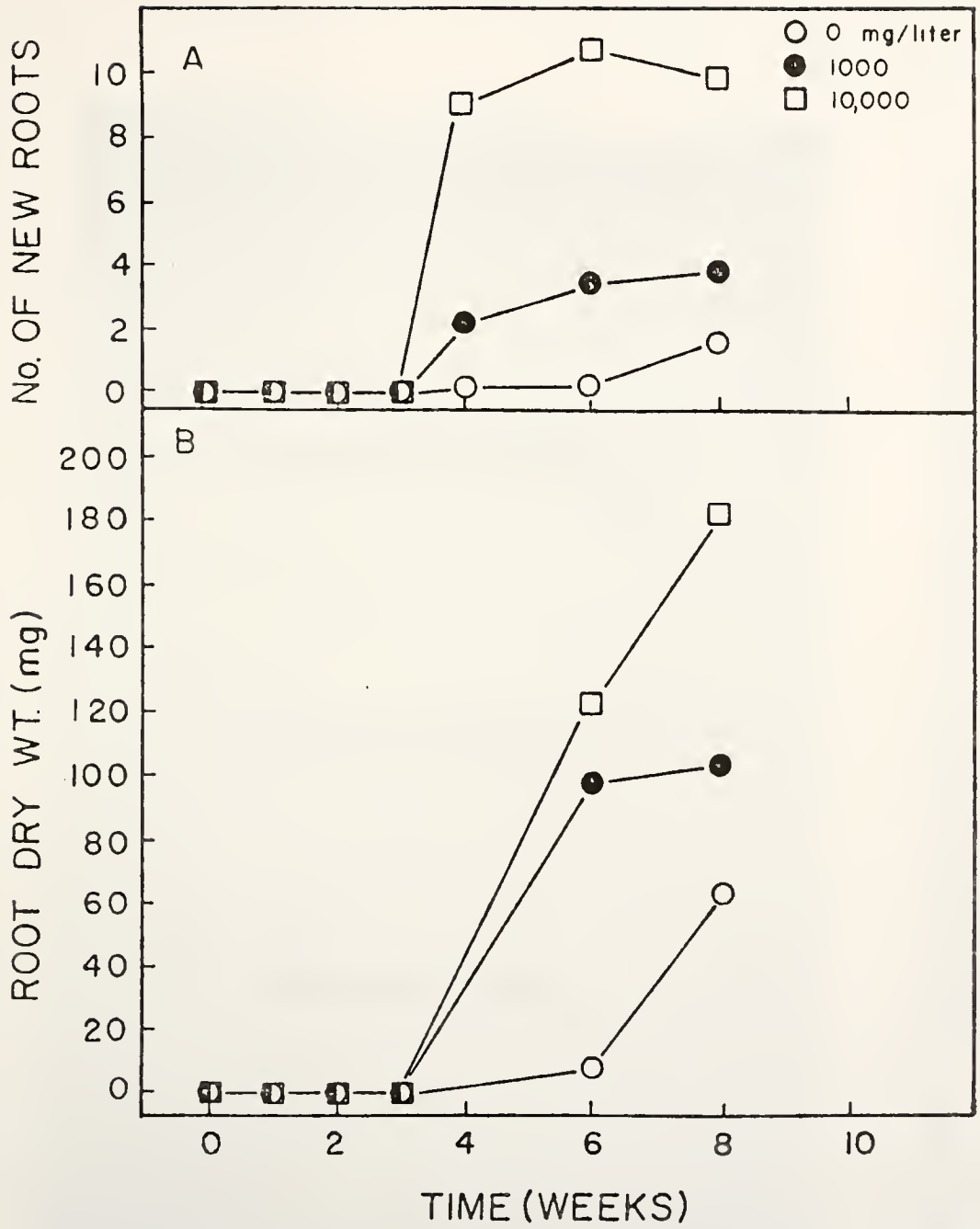

Figure III-1. The number (A) and dry weight (B) of roots initiated on the taproot of tulip tree treated with 0, 1000 and 10,000 mg IBA/liter and harvested at various times.

Figure III-2. Response of tulip tree to increasing concentrations of IBA 8 weeks after transplanting. From left to right: 0, 1000, and 10,000 mg IBA/liter.

declined 50% to 3.34 mg/100 mg DW by 2 weeks, increased slightly by 6 weeks and declined to 2.49 mg/100 mg DW at 8 weeks. The largest decrease in sucrose for all harvest dates occurred when seedlings were treated with 10,000 mg/liter IBA. Sucrose declined to 2.07 mg/100 mg DW by 8 weeks which was 32% of the level measured at the start of the experiment. The amount of sucrose contained in IBA treated plants at 2 weeks was comparable to the amount contained in untreated plants at 8 weeks.

Initially, reducing sugars composed 2% of total dry weight and 7% (2.01 mg/100 mg DW) of total carbohydrates contained in the taproot. There was sufficient variability in the levels of reducing sugars among root sections to result in low r^2 values for most treatments. For the 0 and 1000 mg/liter treatments, reducing sugars declined 34% to 1.33 mg/100 mg DW by 8 weeks. Treating seedlings with 10,000 mg/liter resulted in a trend of increased levels of reducing sugars apparent at 2 weeks. The proximal and medial sections retained the elevated levels of reducing sugars for the duration of the experiment. Reducing sugars in the distal section declined at 6 weeks to levels similar to the 0 and 1000 mg/liter treatments.

Starch content was 27 fold less in the shoots as compared to the roots (0.80 mg/100 mg shoots versus 21.75 mg/100 mg root). There was no significant difference in the rate of starch decline between IBA treatments, and for all treatments starch level decreased 50% by 8 weeks. Sucrose level in the stem was about 1.5 mg/100 mg DW lower than in the taproot at the start of the experiment. Regardless of treatment, sucrose declined 40% to 2.02 mg/100 mg DW by 8 weeks. Reducing sugars were present in the stem tissue at comparable levels with the taproot. IBA treatment did not significantly affect the levels of reducing sugars in the stem which declined 45% to 1.30 mg/100 mg DW by 8 weeks for all treatments.

Carbohydrate analysis of new roots taken from plants 8 weeks after transplanting showed similar levels of sucrose and reducing sugars among IBA treatments (Table III-1). Starch content for these roots declined with increasing IBA treatment.

Discussion

The enhancement of root regeneration by auxin is well known. Much of the research in the area of root regeneration and carbohydrate utilization has been conducted with stem cuttings. Studies have shown that root formation is induced at the site of auxin accumulation (4,11). Carbohydrates transported from the leaves have been shown to be the limiting factor in rooting stem cuttings of Phaseolus vulgaris (1). In addition, labeled assimilates accumulated at the site of root formation. A low carbohydrate content in stem tissue has also been shown to reduce root regeneration in cuttings (6).

In this study, roots were regenerated on dormant plants without a photosynthesizing capacity. Root initiation occurred predominantly at the distal section of the taproot suggesting a preferential utilization of carbohydrates during the course of root regeneration. Endogenous levels of starch and sucrose measured in the taproot of dormant tulip trees decreased during the course of root initiation and elongation. Applications of IBA to the taproot stimulated root regeneration and resulted in larger decreases in starch and sucrose levels in the taproot. Reducing sugars tended to be higher in the taproot of those plants exhibiting increased rooting.

Although there was no significant difference in carbohydrate level within root sections, carbohydrates tended to be lower in the taproot in the distal section; the section where roots were initiated. However, it appears that the decrease in starch and sucrose levels occurs throughout the taproot during the course of root regeneration. Starch accounted for 72% of the total carbohy-

Table III-1. The level of starch, sucrose and reducing sugars contained in roots initiated on dormant tulip trees treated with increasing concentrations of IBA and harvested 8 weeks after transplanting.

Treatment mg IBA/liter	Starch mg/100 mg DW	Sucrose mg/100 mg DW	Reducing sugars mg/100 mg DW
0	33.34	1.82	1.78
1000	10.32	1.63	1.60
10,000	5.00	1.63	2.22

drate measured in the taproot and declined most significantly during the process of root regeneration. Root regeneration was apparent at 4 weeks after transplanting. Reductions in starch and sucrose level were observed 2 weeks after transplanting which was well in advance of visible root regeneration. Starch was heavily depleted in plants treated with 10,000 mg/liter which may result in reduced vigor when plants resume growth in the spring.

Root initiation requires energy with the source provided by the degradation of carbohydrates. Starch content of stem cutting has been shown to decline rapidly during root initiation (2,5). The free sugar content in the lower stem of cuttings usually increases during the early stages of root initiation and development because of starch hydrolysis and basipetal translocation (5). This could explain the increased levels of sucrose in the 1000 mg/liter and reducing sugars in the 10,000 mg/liter treatments.

While the levels of starch, sucrose, and reducing sugars in the stem tissue declined during root regeneration, there was no significant difference between IBA treatments. The total amount of carbohydrates in the shoot was also considerably less than the taproot, and it appears the carbohydrates in the stem do not make up a significant portion of those utilized directly for root regeneration. These results indicate that root regeneration in dormant tulip tree seedlings is dependent primarily on the carbohydrates contained in the taproot with starch as the major source.

The role of the dormant shoot in adventitious root formation on root tissue is unclear. The shoot appears to exert some control in the root regeneration process both as a supplier of carbohydrates and growth regulators. Disbudded seedlings (7) and root cuttings (Kelly and Moser, unpublished data) of dormant tulip tree root poorly. Similar reductions in root regeneration have been observed with disbudded Acer saccharinum, (9) thereby supporting the theory

that some endogenous control of root regeneration is located in the shoot. The influence of the shoot on root regeneration is probably hormonal; however, this remains to be verified.

Starch and sucrose content of the taproot and shoot declined during root regeneration in dormant tulip tree which implies significant biochemical changes are occurring within the plant. Nucleic acids and proteins increased during rooting of stem cuttings of Populus nigra, and protein synthesis was shown to be essential for rooting (8). Little information is available on the biochemical events associated with root initiation in roots and the subsequent growth of the newly initiated root.

Plant growth early in the season depends to a large extent on stored carbohydrate reserves. The utilization of starch for root regeneration highlights the importance of healthy transplant with optimum carbohydrate reserves. Since carbohydrates are greatly reduced by 8 weeks in 10,000 mg/liter treatment and the newly initiated roots contain less starch suggests that this treatment may stimulate more roots than can adequately be supported by the carbohydrate reserve contained in the plant. Reduced carbohydrate levels which result from root regeneration in the dormant may influence the long-term growth of these plants, but remains to be determined. When tulip tree is transplanted in the spring, shoot growth proceeds before root regeneration (7). This could change the pattern of carbohydrate utilization during the course of root regeneration and needs further investigation. Understanding the physiological processes which occur during root regeneration will be useful in selecting cultural practices to enhance survival and growth of transplants.

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CONCLUSIONS AND APPLICATIONS

The following conclusions and applications can be directly applied to the establishment of bare root trees on the roadside. This work is applicable to those species that are difficult to establish and have low survival rates.

1) Root systems at the nursery source should be of a well branched nature - auxin treatments and cultural practices can assure this, i.e. used in the production cycle.

2) Auxin preplant treatments hasten the regeneration of a new root system and should be considered for coarse rooted bare root material that is known to be difficult to transplant.

3) Auxin treatment has no beneficial effects on easy to transplant fibrous rooted species and is not recommended.

4) Auxin treatment is detrimental to pines and should not be used.

5) Seasonal responses in root regeneration should be considered in scheduling highway plantings.

6) Use of auxins in fall transplants is not recommended.

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